RESEARCH PROPOSAL:

Morphogenesis of higher brain functions by the selective action of the transient subplate layer

A SERIES OF EXPERIMENTS ON CAT AREA 18

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Introduction

During development of the neocortex in rats (Wise & Jones, 1978), in cats (Luskin & Shatz, 1985; Chun *et al.*, 1987), and in humans (Ladislav *et al.*, 1988) a large, transient layer of neurons appears, the subplate, in which ingrowing axon growth cones accumulate diffusely and produce transient synapses (Molliver *et al.*, 1973) for weeks before any of them penetrate the cortical plate above. For both thalamic and callosal innervation, those axons that penetrate the cortical plate will be retained in the fully differentiated cortex, while those that do not will be eliminated (Innocenti, 1981). Penetration coincides with disappearance of the subplate, suggesting a selective action by the subplate on innervation patterns (Chun *et al.*, 1987). Contalateral callosal axons terminate in the subplate in conjunction with fast synapse proliferation, which is followed by synchronous axon colateral elimination postnatally, further followed by the final pattern formation of the originating contralateral callosal perikarya. Every neuron that lost its contralateral projection retains or initiates permanent ipsilateral projections. The final distribution of the callosal perikarya may thus be largely determined by their contralateral connections (Innocenti, 1981), and hense determined to a large degree by the transient subplate layer (review: Innocenti, 1988). Upper subplate cells contain somatostatin while lower subplate cells contain neuropeptide Y (Chun *et al.*, 1987) and correlate spacially and temperally with a fibronectin-like substrate (FNi) (Chun & Shatz, 1988). Morphogenesis is known to act by substrate recognition or adhesion, and possibly by transient neurotransmitter expression (Parnavelas & Cavenagh, 1988), so these subplate substances may play a key role in determination of interhemisheric connections.

The most salient feature of adult cortical projections that might be induced by the transient subplate is their tangental pattern. Their pattern, both intrinsic and callosal, consists of patches of a few dozen perikarya in roughly 1-mm periods, as shown, for example, by Goldman-Rakic and Shwartz (1982) in monkey prefrontal cortex, and by Matsubara *et al.* in cat visual cortex for both intrinsic (1987) and callosal (personal communication) projections (which are restricted to the vertical meridian). The studies of callosal projections using only horseradish peroxidase (HRP) do not indicate whether retrogradely-labeled patches also project back to the same contralateral injection site, as is largely assumed, and in fact Imig & Bruge (1978) showed a mismatch (in auditory cortex). This reciprocal relationship for the corpus callosum is now being determined for cat area 18 by Matsubara and Boyd at U.B.C. by the addition of strictly anterograde tracers.

As yet there has been no direct test of whether the transient subplate affects the arrangement of callosal patches. If it does, does it act via synaptic transmission, contact guidance, substrate molecules, or a combination?

Proposed Experiment

The subplate of the vertical-meridian area of cortical area 18 in cat embryos will be manipulated and each animal will be evaluated postnatally for its tangental cortical pattern of callosal perikarya and callosal axon terminations, bilaterally. 48 cats will form 12 groups in a 3 by 2 by 2 factorial design. Sham operations will be made in a different part of the vertical-meridian area so that each animal serves as its own control. The design is as follows:

(A) Three lesion types:

- o A1: inject neurotoxin just as the subplate becomes established (disrupting transmission, structure, and FNi presence).
- o A2: inject neurotoxin only when the axons begin to arrive (stopping transmission, but little affect on structure and FNi).
- o A3: inject anti-FN antibody just as axons begin to arrive (blocking adhesion or recognition, but not subplate-cell function or structure).

(B) All three lesion types will be made in both upper-subplate and lower-subplate groups.

(C) Dependent variables will be observed both at birth and in adults.

Cat visual cortex was chosen because its callosal projections are the most well characterized anatomically (Voight *et al.*, 1988; Berbel & Innocenti, 1988) and functionally (Matsubara, personal communication). Group size should allow quantification and analysis of variance in the case that developmental alterations are not dramatic.

Materials and Methods

1. Intrauterine injections: By pulse-chase labeling with intrauterine [3H]thymidine on the subplate cells' postmitotic birthdate at embryonic day 27 (E27), autoradiography can later be used to identify the subplate in evaluation of brain injections (Luskin & Shatz, 1985).

2. Fetal subplate microinjections: See Chun & Shatz (1988) for subplate microinjection and surgery procedures. Kainic acid (Coyle *et al.*, 1978) will be injected in E35 and E45 fetuses (A1 & A2) (Chun & Shatz, 1988). Since antibodies can block FN recognition and thereby disrupt neural migration in vivo (Brenner-Fraser, 1986) we will microinject another E45 group with commercially produced rabbit anti-human FN serum (A3), which binds to the cat FNi (Chun & Shatz, 1988). Ages are determined by timed breeding (Luskin & Shatz, 1985).

3. Postnatal labeling: Bilateral, tangental projection patterns will be traced in both hemispheres at birth as well as on postnatal day 150 (P150) in order to discriminate between (a) initial disruption of afferent terminals, and (b) disruption of callosal perikarya pattern-formation following axon elimination. The same retrograde wheatgerm agglutinin (WGA)-HRP methods used by Matsubara *et al.* (1987) for intrinsic projections will be combined with anterograde staining using phaseolus vulgaris (Gerfen & Sawchenko, 1984). An array of both fetal lesion sites

and of contralateral retro- and anterograde tracer-injection sites will ensure that a match can be obtained. Lesion sites can be examined in coronal sections by autoradiographic silver-grain distribution and by anti-FN staining, but to view all remaining cells we will also stain with anti-microtubule-associated protein 2. For tissue preparation and immunohistochemistry see Chun & Shatz (1988).

Potential Results and Subsequent Experiments (Abridged)

If no effect: look next for cortical trophism or selective chemotaxis. For any effect: repeat with (a) functional mapping by electrophysiological recording prior to contralateral HRP (Matsubara 1987), and/or (b) in areas such as V4, inferior temporal, and prefrontal (which have less topographical mapping). Effect from lesion A1 only : implies contact guidance. Effect from lesion A2 only: implies neurotransmission is neccessary; try chronic fetal tetrodotoxin or manganese (add dye at end to locate). Effect from lesions A3 and A1 (but not A2): substrate molecules are needed and synaptic action is incidental. Effect from lesion A2 and A3 (not A1): neural transmission is needed in addition to FNi or as a cause of FNi distribution. In general: Actions of the upper and lower subplate may be independent or dependent, by the same or different mechanisms, or only one may act. Any effect at P150 on contralateral perikarya supports the hypothesis that pattern formation is determined by the terminations contralaterally (i.e., by the lesion site), while an ipsilateral perikarya effect implies an additional mechanism, affected by the subplate. Terminations and/or perikarya may remain diffuse, or termination patches may separate from perikarya patches. If for control lesions or within a lesion group an innervation pattern of terminations at birth does not correspond to adult patches, then selection by the subplate is not directly for patch pattern. This experiment may clarify the degree of precision of normal homotopic reciprocal projections, as well as their morphogenesis. If axon elimination fails to occur, we will then have an excellent preparation for detailed anatomical, functional, and behavioural studies of the affects of callosal axon elimination and of callosal processing in the adult.

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